Arquivos do Museu Nacional, Rio de Janeiro, v.64, n.4, p.325-332, out./dez.2006
ISSN 0365-4508

MORPHOMETRIC DISCRIMINATION BETWEEN *TRINOMYS ALBISPINUS* (IS. GEOFFROY, 1838) AND *TRINOMYS MINOR* (REIS & PESSÔA, 1995) FROM CHAPADA DIAMANTINA, BAHIA, BRAZIL, AND THE KARYOTYPE OF *TRINOMYS ALBISPINUS* (RODENTIA, ECHIMYIDAE) [1]

(With 5 figures)

ANA LAZAR GOMES E SOUZA [2]
MARGARET MARIA DE O. CORRÊA [2]
LEILA MARIA PESSÔA [2,3]

ABSTRACT: A morphometric discrimination analysis was performed for *Trinomys minor* (Reis & Pessôa, 1995) and *Trinomys albispinus* (Is. Geoffroy, 1838). The samples used in this study are from localities in the Chapada Diamantina, a vast plateau in central Bahia State, Brazil. A specimen recently obtained near the type-locality of *Trinomys minor* was allocated to *T. albispinus* by principal component and discriminant analyses and by qualitative pelage traits. The karyotype of *Trinomys albispinus* is described on the basis of this specimen as 2n=60, NA=116, with two Nucleolar Organizer Regions (NORs) located in the interstitial region of the long arm of chromosome pair 10. The similarity between this karyotype and that previously published for *T. minor* is interpreted here as evidence that *T. minor* and *T. albispinus* are closely related forms, probably at subspecific level. A pattern of karyological similarity is here documented for other species pairs in the genus in which a close relationship has been revealed by mitochondrial DNA data.

Key words: *Trinomys albispinus*. *Trinomys minor*. Morphometry. Cytogenetic data. Chapada Diamantina.

RESUMO: Discriminação morfométrica entre *Trinomys albispinus* (Is. Geoffroy, 1838) e *Trinomys minor* (Reis & Pessôa, 1995) da Chapada Diamantina, Bahia, Brasil, e o carioótipo de *Trinomys albispinus* (Rodentia, Echimyidae).

Uma análise de discriminação morfométrica foi realizada entre *Trinomys albispinus* (Is. Geoffroy, 1838) e *Trinomys minor* (Reis & Pessôa, 1995) com base em amostras provenientes da Chapada Diamantina, um vasto platô situado na área central da Bahia. Um espécime recentemente obtido próximo à localidade-tipo de *T. minor* foi alocado em análises morfométricas multivariadas e em comparações da pelagem à *T. albispinus*. O cariótipo de *Trinomys albispinus* é descrito com base neste espécime. *Trinomys albispinus* apresentou 2n= 60 e NA= 116, e duas regiões organizadoras de nucléolo (RONs) localizadas na região intersticial do braço longo do par cromossômico 10. A similaridade cromossômica entre esse cariótipo e o previamente publicado para *T. minor* é interpretada aqui como evidência que *T. minor e T. albispinus* são espécies muito relacionadas, provavelmente em nível subespecífico. Um padrão de similaridade cariotípica é aqui documentado entre outros pares de espécies no gênero onde uma relação filogenética próxima tenha sido revelada por análises de DNA mitocondrial.

Palavras-chave: *Trinomys albispinus*. *Trinomys minor*. Morfometria. Dados citogenéticos. Chapada Diamantina.

INTRODUCTION

Moojen (1948), in the first revision of *Proechimys* (*Trinomys*) *albispinus* (Is. Geoffroy, 1839), examined population samples from the state of Bahia in northeastern Brazil and recognized two subspecies. According to Moojen (1948), *P. albispinus albispinus* (Is. Geoffroy, 1839) has a narrow skull with orthodont incisors and a darker coloration on the sides of the body, with clavate aristiform hairs in the mid-dorsal region and on the outer thighs, always with an Ochraceous-Tawny subapical zone. In *P. albispinus sertonius* (Thomas, 1921) the skull is broad with proodont incisors and the pelage has mostly clavate aristiform hairs with two color patterns, one characterized by the presence of an Ochraceous-Tawny subapical zone and the other blackening toward the tip. The aristiform hairs on

[1] Submitted on July 11, 2005. Accepted on august 3, 2006.
[2] Universidade Federal do Rio de Janeiro, IB, Departamento de Zoologia. 21941-590, Rio de Janeiro, RJ, Brasil.
[3] Fellow of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).
E-mail: pessoa@acd.ufrj.br

the outer thighs have a Light Ochraceous-Buff subapical zone (Moojen, 1948). The sample analyzed by Moojen was restricted to nine specimens of *P. a. albispinus* from Macaco Seco, Andaraí, Chapada Diamantina, and four of *P. a. sertonius*, one being from Lamarão and three from Bonfim, Bahia. In recent years, further specimens became available from localities in the states of Sergipe, Bahia and northern Minas Gerais, allowing new analyses of geographic distribution and morphological variation (Pessôa & Reis, 1991; Pessôa & Reis, 1992; Pessôa & Reis, 1995; Pessôa; Von Zuben & Reis, 1998, Pessôa & Strauss, 1999; Pessôa & Reis, 2002).

Cytological work was performed by Leal-Mesquita *et al.* (1992), who described the karyotype (2n=60, NA=116) of specimens collected at Morro do Chapéu, Bahia, and attributed the name *Proechimys albispinus* to this sample. Subsequently, Reis & Pessôa (1995) re-examined this material, compared it with new specimens collected at the same locality, and described it as a new subspecies of *P. albispinus*, namely *Proechimys albispinus minor* Reis & Pessôa, 1995. Its type-locality was Morro do Chapéu (11°33'S 41°09'W), Bahia, at 1000 m.

In a study of cranial variation in *P. albispinus*, Pessôa & Strauss (1999) showed that *P. a. albispinus* and *P. a. sertonius* overlap widely in their morphological distributions, differing only on averages of greatest skull length. On the other hand, almost complete discrimination between *P. a. minor* and each of the other two subspecies based on cranial shape was documented.

Iack-Ximenes (2005) examined population samples from Sergipe, Bahia and northern Minas Gerais States, compared them with the type material of *P. a. albispinus* and *P. a sertonius*, and concluded that it was impossible to recognize morphological distinction between these subspecies. He regarded *T. sertonius* as a synonym of *Trinomys albispinus* and suggested that *Trinomys albispinus minor* should be raised to species rank.

Until 2005, information on chromosomal variation had been published only for three of the thirteen recognized taxa of *Trinomys*, namely *T. minor* (2n=60, NA=116), from Morro do Chapéu (Leal-Mesquita *et al.*, 1992), *T. yonenagae* (Rocha, 1995) (2n=54, NA=108) from Ibiraba, also in Bahia State, and *T. iheringi* (Thomas, 1921) (2n=61-65, NA=116), from several coastal localities in São Paulo State (Yonenaga-Yassuda *et al.*, 1985; Leal-Mesquita *et al.*, 1992). Recently, chromosome morphology and chromosomal complement were characterized for five additional *Trinomys* taxa, namely *T. dimidiatus* (Gunther, 1876) (2n=60, NA=116); *T. gratiosus bonafidei* (Moojen, 1948) (2n=56, NA=108), *T. eliasi* (Pessôa & Reis, 1993) (2n=58, NA=112) (Pessôa *et al.*, 2005); *T. setosus elegans* (Lund, 1841) (2n=56, NA=104) and *T. moojeni* (Pessôa, Oliveira & Reis, 1992) (2n=56, NA=106) (Corrêa et *al.*, 2005).

The phylogenetic relationships among *Trinomys albispinus* and other taxa within the genus were elucidated by Lara & Patton (2000), who proposed three major clades within *Trinomys* based on the analysis of cytochrome *b* genetic data. One clade included the monotypic taxa *T. dimidiatus*, *T. iheringi*, and *T. mirapitanga*, and the polytypic *T. gratiosus*; a second clade included *T. yonenagae*, *T. paratus*, *T. eliasi*, and the polytypic *T. setosus*; a third basal clade was represented only by *T. albispinus*.

We recently collected one specimen of *T. albispinus* at Cachoeira do Ferro Doido, a locality 30 km from the type-locality of *T. minor*, also in the municipality of Morro do Chapéu (Fig.1). This specimen differs from *T. minor* by its larger body and cranial size and its wider aristiform hairs, which have an Ochraceous-Tawny subapical zone in the mid-dorsal region and a Light Ochraceous-Buff subapical zone on the outer thighs. Since these qualitative morphological characters are not easy to recognize, we used morphometric methods to allocate this recently collected specimen to previously identified samples of *Trinomys albispinus* or *T. minor*, as described below. We then describe the karyotype of *T. albispinus* based on this specimen, comparing it with the karyotype of *T. minor* described by Leal-Mesquita *et al.* (1992). Finally, we discuss the similarity between karyotypes of *T. albispinus* and *T. minor* in the context of karyological variation and phylogenetic relationships in the genus, as previously revealed by cytochrome *b* genetic data.

MATERIAL AND METHODS

A total of 32 adult specimens from Chapada Diamantina was analyzed in the present study. Adult specimens were selected following Pessôa & Reis (1991). Eight specimens of *Trinomys minor* (MN67763, MN67773, MN67774, MN69795, MN69796, MN69797, MN69798, MN69799) were recently collected at Fazenda Jaboticaba and Morrão, both localities situated in the municipality of Morro do Chapéu. Morrão is actually the site where the type series of *T. minor* was obtained.

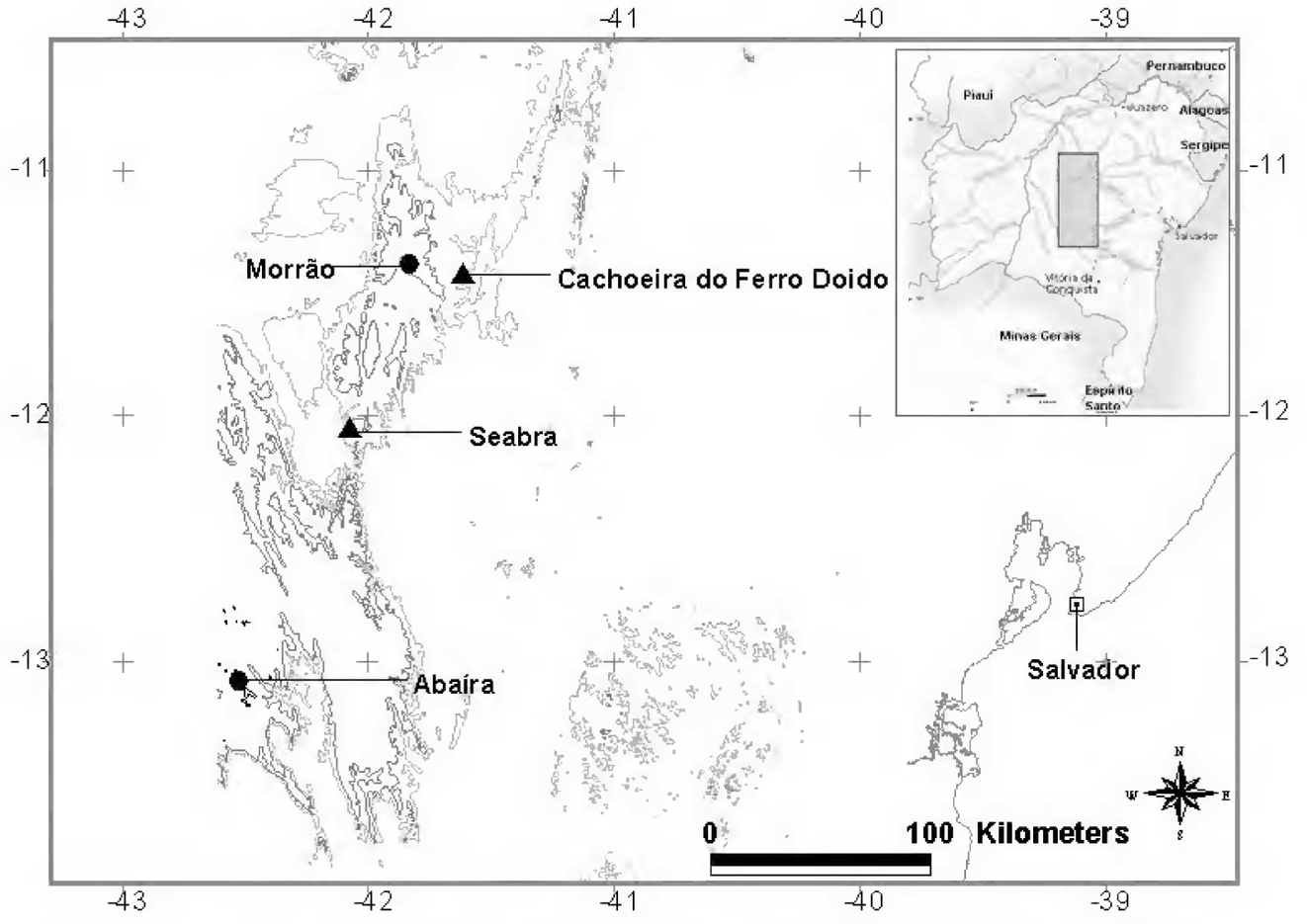

Fig.1- Map of the state of Bahia showing the localities of samples used in this study, and with contour lines delimiting altitudes of 800m (clear gray) and 1200m (dark gray) in the Chapada Diamantina.

Also included in the analysis were the three specimens from the type series of *T. minor* (MN44546, MN44543, MN34491) and one additional specimen (MN67814) recently collected in Catolés de Cima, Abaíra, in the southern part of the Chapada Diamantina. The sample of *T. albispinus* was restricted to 19 specimens from the municipality of Seabra (MN6454, MN13764, MN13768, MN13790, MN13792, MN13793, MN13890, MN13873, MN13882, MN13886, MN13922, MN13923, MN30526, MN34004-MN34007, MN34009 and MN34011). A male specimen of *Trinomys albispinus* (MN67903), recently collected at Cachoeira do Ferro Doido, 30 km from Morrão, was karyotyped, prepared as skin, skull and skeleton, and was also included in the morphometric analysis. The voucher material is lodged in the Mammal Collection of the Museu Nacional, Rio de Janeiro.

Ten cranial measurements, eight of which defined by Patton & Rogers (1983), were obtained: skull length (SL), basal length (BL), zygomatic breadth (ZB), diastema (D), rostral length (RL), nasal length (NL), bulla length (BUL), and toothrow length (TL). The two additional cranial measurements, defined by Smith & Patton (1988), were occipito-nasal length (ONL) and minimal palatal width (MPW).

Cranial character variation in *T. minor* and *T. albispinus* was analyzed by univariate and multivariate statistical procedures, namely Analysis of Variance (ANOVA) (Sokal & Rohlf, 1981), Principal Components Analysis (PCA) and Discriminant Analysis (Manly, 1994). Recently collected specimens that could not be confidently identified by pelage characters were subjected to a classification procedure based on minimum size-adjusted Mahalanobis distances, bootstrapped (1000 iterations) to estimate classification frequency distributions.

Cytogenetic analyses were performed on mitotic metaphase chromosomes from bone marrow following FORD & HAMERTON (1956) with modifications mainly related to a shortening of the exposure to colchicine. Chromosomes were stained with Giemsa and classified according to LEVAN, FREDGA & SANDBERG (1964). Metacentric, submetacentric and subtelocentric chromosomes were considered biarmed, and acrocentric chromosomes, uniarmed. The silver nitrate staining technique followed the procedures of HOWELL & BLACK (1980).

RESULTS

The descriptive statistics of cranial dimensions in *T. minor* and *T. albispinus* are given in table 1. Univariate analysis of variance (ANOVA) indicates significant differences in most characters between the two species ($P<0.001$).

A large amount of the cranial variation sampled by the measurements (> 90%) was summarized in the first principal component of the log-transformed covariance matrix. All characters loaded positively on this component, indicating that it can be interpreted as a size vector. When individual scores were plotted in the multivariate space defined by the first two principal components, an almost complete separation between the samples assigned to *T. minor* and *T. albispinus* was revealed (Fig.2). Discriminant analyses allocated the Catolés de Cima specimen to *T. minor* and the Cachoeira do Ferro Doido specimen to *T. albispinus* in 100% of cases (Tab.2).

Cytogenetic analysis of the *T. albispinus* specimen from Cachoeira do Ferro Doido revealed a diploid number 2n=60 and number of autosomal arms NA=116. This karyotype comprises 29 pairs of biarmed autosomes, 17 of which are metacentric, 7 submetacentric and 5 subtelocentric. The X chromosome is a large submetacentric intermediate in size between pairs 1 and 2. The Y chromosome is a small acrocentric (Fig.3).

Ag-NOR staining showed that there are two NORs located in the interstitial region of the long arm of chromosome pair 10 (Fig.4).

DISCUSSION

PESSÔA & STRAUSS (1999) used multivariate statistical methods to evaluate whether cranial morphometric data supported MOOJEN´s (1948) designation of subspecies in *Proechimys (Trinomys) albispinus*, based on pelage and qualitative aspects of the skull. They found that morphometric analyses only partially supported his designations, because *T. a. albispinus* and *T. a. sertonius* overlapped widely in their morphological distributions, differing only on averages. On the other hand, their analysis revealed a complete discrimination of *T. a. minor* from the two other subspecies on the basis of cranial shape. These authors noted that the small skull and body size, thinner guard hairs in the mid-dorsal region, and the lack of an ochraceous subapical zone on the guard hairs found in adult specimens of *T. a. minor* were similar to the character states found in juveniles of *T. albispinus*. They hypothesized that these differences were due to heterochronic changes during the evolution of these species.

TABLE 1. Mean, standard-deviation (SD) and *F*-values and associated probabilities (*p*) from analyses of variance of 10 cranial measurements taken from samples of *T. minor* and in *T. albispinus*.

CHARACTER	*T. minor* (n=12)		*T. albispinus* (n=20)			
	MEAN	SD	MEAN	SD	*F*	*p*
GSL	42.639	1.583	46.503	1.325	55.13	< 0.001
BL	31.68	0.911	35.407	1.338	72.47	< 0.001
ONL	39.578	1.282	43.501	1.486	57.66	< 0.001
MPW	3.2508	0.282	3.782	0.3019	24.36	< 0.001
DL	10.163	0.391	11.67	0.643	53.5	< 0.001
ZB	22.867	0.411	24.33	0.825	32.53	< 0.001
NL	14.576	0.76	16.139	0.79	29.86	< 0.001
RL	15.974	1.424	16.669	0.703	3.43	0.074
BUL	10.994	0.457	11.867	0.334	38.76	< 0.001
MTRL	7.0217	0.3074	7.469	0.2415	20.96	< 0.001

Our cytogenetic analysis of an individual of *T. albispinus* shows that it does not differ from *T. minor* in diploid and autosomal numbers, morphology of the sex chromosomes, or size of the first and second pairs of autosomes. The Ag-NOR sites of *T. albispinus* are also similar to those published for *T. minor* by LEAL-MESQUITA *et al.* (1992).

Karyotypic similarity has been documented for species pairs in the genus (Fig.5): *T. dimidiatus* and *T. iheringi* have the same diploid and autosomal numbers and a similar X chromosome, although the Y chromosome differs between the species. A pattern of similar diploid and autosomal numbers also occurs between the two subspecies of *T. gratiosus*. Both these pairs have been shown on the basis of cytochrome *b* gene sequences to comprise closely related forms (LARA & PATTON, 2000). Conversely, a deeper divergence in cytochrome *b* sequences, as detected between *T. yonenagae* and *T. eliasi*, for instance, is coincident with a greater number of diploid and autosomal changes (Fig.5). This pattern is interpreted in the case of *T. albispinus* and *T. minor* as indicative that these forms have diverged recently or are in fact in a process of divergence, a condition that would justify their taxonomic distinction at subspecific level.

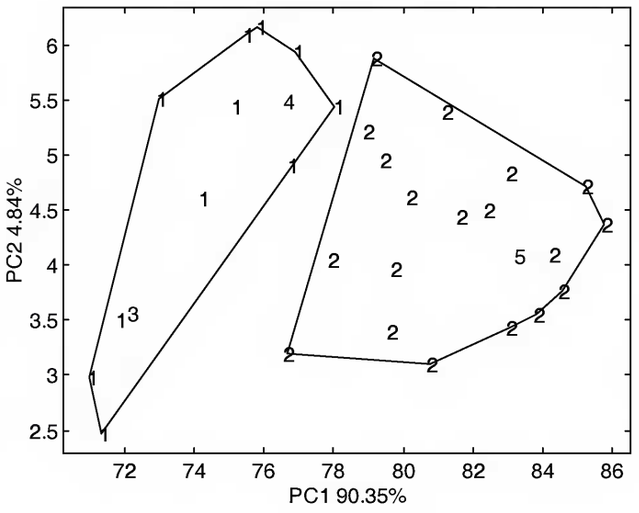

Fig.2- Plot of Principal Component (PC1 x PC2) individual scores of all specimens labeled in reference to their localities: 1) Specimens identified as *T. minor*, from "Morrão"; 2. Specimens identified as *T. albispinus*, from Seabra; 3. The specimen of *T. minor* from Catolés de Cima, Abaíra; 4. An unidentified specimen from "Morrão"; 5. The specimen of *T. albispinus* from "Cachoeira do Ferro Doido", Morro do Chapéu.

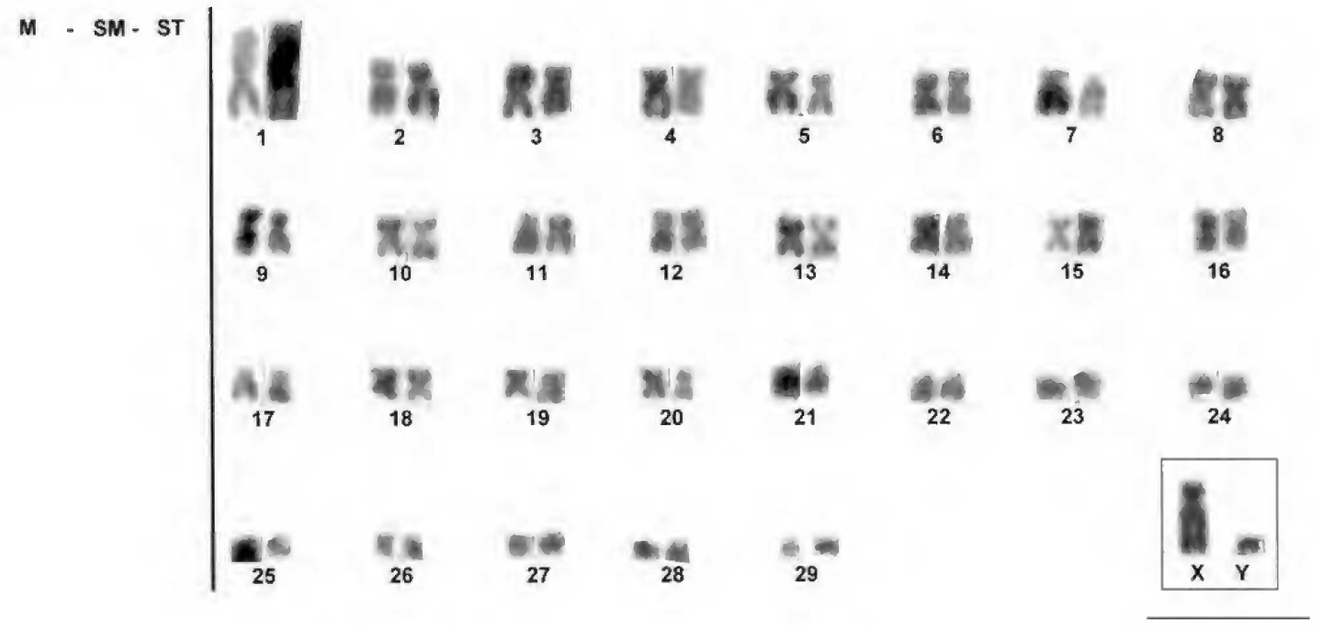

Fig.3- Karyotype of a male individual of *Trinomys albispinus* (2n = 60, NA = 116) from Cachoeira do Ferro Doido, Morro do Chapéu, Bahia. Scale bar = 10μm.

TABLE 2. Percentage and (number of individuals) correctly allocated to *T. albispinus* and *T. minor* by a discriminant function analysis.

	PREDICTED GROUP	
Actual group	*T. minor*	*T. albispinus*
T. minor	100% (11)	
T. albispinus		100% (19)

The implications and relevance of these new data on chromosomal morphology for the taxonomy and systematics of *Trinomys* have been evaluated in the context of the taxonomic structure derived from molecular data. It should be noted that available karyological information and distribution records are still limited in the genus.

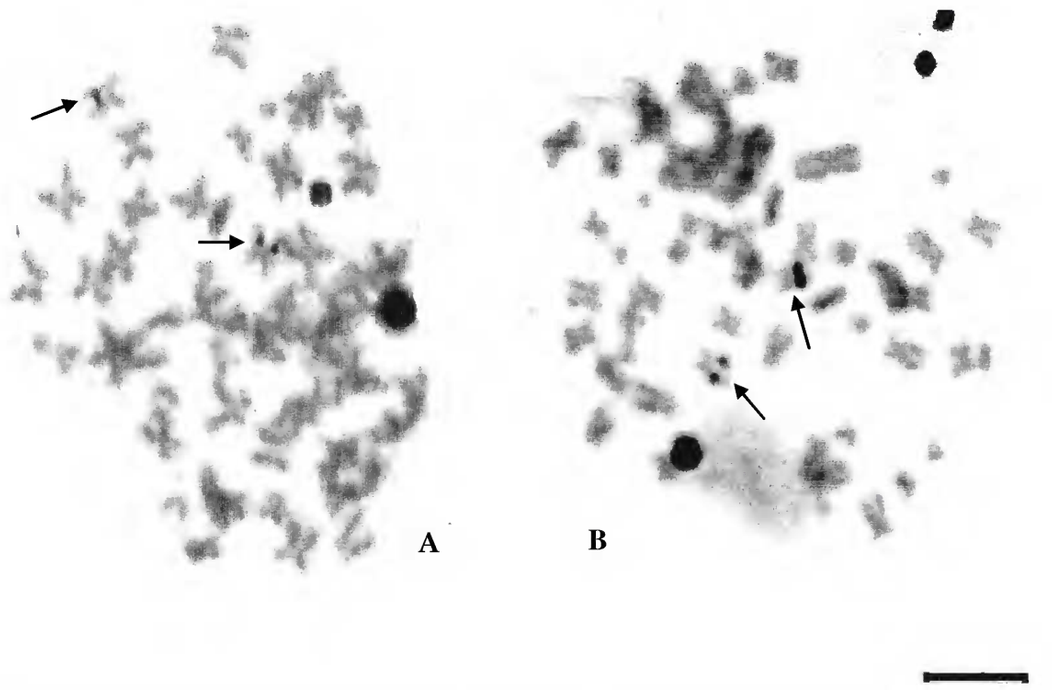

Fig.4- a-b) Metaphase cells treated with silver nitrate staining. The arrows indicate Ag-NOR sites located interstitially on the long arm of pair 10. Scale bar = 10μm.

ACKNOWLEDGEMENTS

The authors thank the curators at the Museu Nacional, Rio de Janeiro, for allowing access to specimens. Stella M. S. Franco kindly helped with the handling of specimens. We are grateful to José Aloisio Brandão Cardoso (Centro de Recursos Ambientais, Morro do Chapéu), for his support and interest during fieldwork. We are indebted to C.J.Tribe (University Museum of Zoology, Cambridge) for reviewing the English of a previous version of this manuscript. This work was funded by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, PROBIO/Chapada Diamantina, Biodiversidade) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). IBAMA issued permits for the collection of specimens examined in this work.

REFERENCES

CORRÊA, M.M.O.; GARCIA-LOPES, M.O.; CÂMARA, E.V.C.; OLIVEIRA, L.C., & PESSÔA L.M., 2005. The karyotype of *Trinomys moojeni* (Pessôa, Oliveira & Reis, 1992), and *Trinomys setosus elegans* (Lund, 1841), (Rodentia: Echimyidae), from Minas Gerais, eastern Brazil. **Arquivos do Museu Nacional**, Rio de Janeiro, **63**:169-174.

FORD, C.E. & HAMERTON, J.L., 1956. A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. **Stain Technology**, Louisville, **31**:247-251.

HOWELL, W.M. & BLACK, D.A., 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. **Experientia**, Basel, **36**:1014-1015.

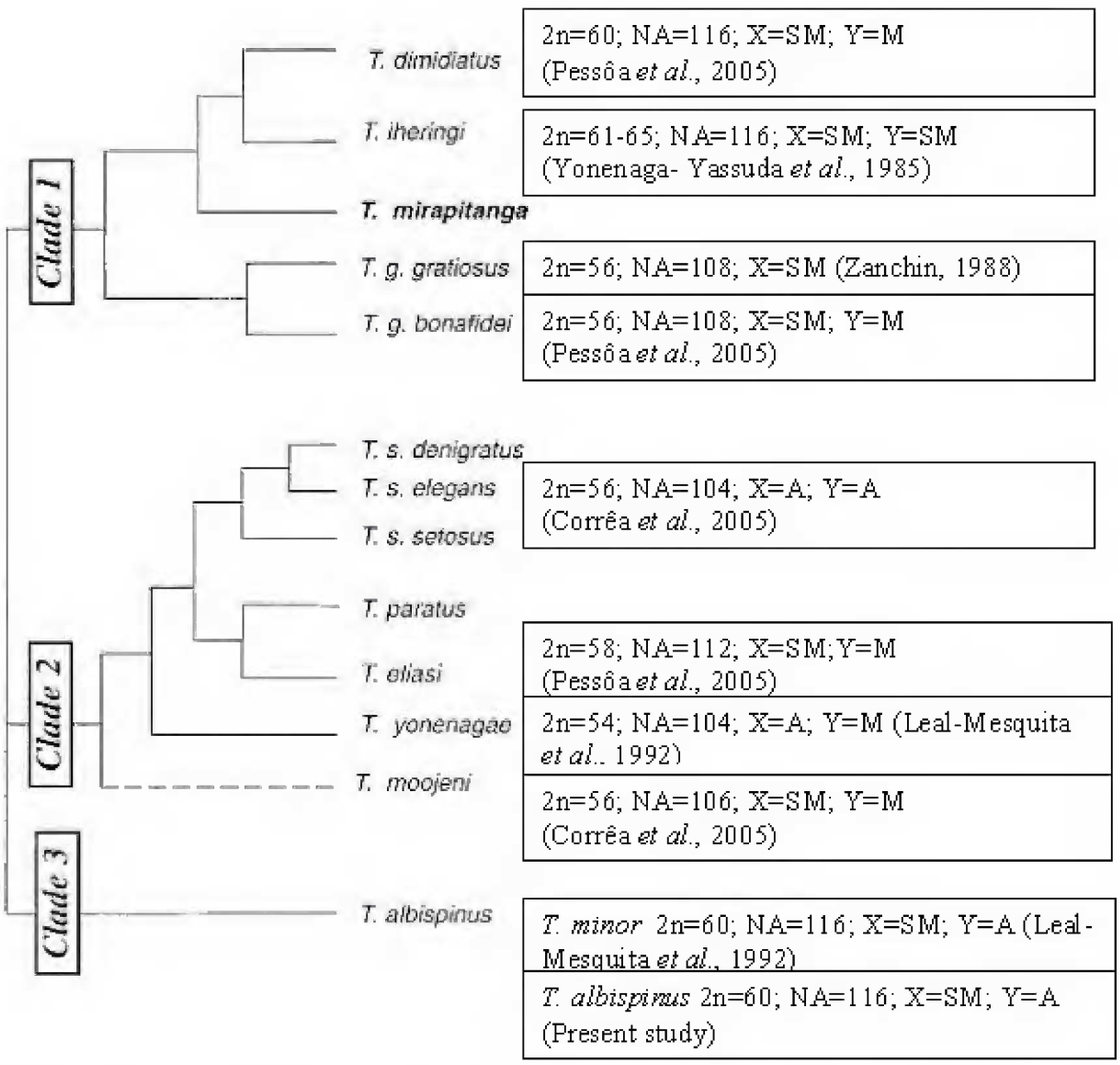

Fig.5- Molecular (cytochrome *b* gene) cladogram of the genus *Trinomys*, as proposed by Lara & Patton (2000), and corresponding karyotypes of the species studied to date.

IACK-XIMENES, G.E., 2005. **Sistemática de *Trinomys* Thomas, 1921 (Rodentia, Histricognathi, Echimyidae).** Unpublished PhD dissertation, Instituto de Biociências, Universidade de São Paulo, São Paulo, 522p.

LARA, M.C. & PATTON, J.L., 2000. Evolutionary diversification of spiny rats (Genus *Trinomys*, Rodentia: Echimyidae) in the Atlantic forest of Brazil. **Zoological Journal of the Linnean Society**, London, **130**:661-686.

LEAL-MESQUITA, E.R.; YONENAGA-YASSUDA, Y.; CHU, T.H. & ROCHA, P.L.B., 1992. Chromosomal characterization and comparative cytogenetic analysis of two species of *Proechimys* (Echimyidae, Rodentia) from the Caatinga domain of the State of Bahia, Brazil. **Caryologia**, Florence, **45**(2):197-212.

LEVAN, A.; FREDGA, K. & SANDBERG, A.A., 1964. Nomenclature for centromeric position on chromosomes. **Hereditas**, Lund, **52**:201-220.

MANLY, B.F.J., 1994. **Multivariate Statistical Methods – A primer**. Second Edition. London: Chapman & Hall. 215p.

MOOJEN, J., 1948. Speciation in the Brazilian spiny rats (Genus *Proechimys*, Family Echimyidae). **University of Kansas Publications of the Museum of Natural History**, Lawrence, **1**:301-406.

PATTON, J.L. & ROGERS, M.A., 1983. Systematic implications of non-geographic variation in the spiny rats genus Proechimys (Echimyidae). **Zeitschrift für Säugetierkunde**, Jena, **48**:363-370.

PESSÔA, L.M., & REIS, S.F., 1991. The contribution of indeterminate growth to non-geographic variation in adult *Proechimys albispinus* (Echimyidae). **Zeitschrift für Säuegetierkunde**, Jena, **56**:219-224.

PESSÔA, L.M. & REIS, S.F., 1992. Bacular variation in the subgenus *Trinomys*, genus *Proechimys* (Rodentia: Echimyidae). **Zeitschrift für Säugetierkunde**, Jena, **57**:100-102.

PESSÔA, L.M., & REIS, S.F., 1995. Coat color variation in *Proechimys albispinus* (Geoffroy, 1838) (Rodentia, Echimyidae). **Boletim do Museu Nacional, Nova Série, Zoologia** (361):1-5.

PESSÔA, L.M.; VON ZUBEN, F.J. & REIS, S.F., 1998. Morphologic affinities of *Proechimys yonenagae* (Rodentia: Echimyidae): evidence from bacular and cranial characters. **Bonner Zoologische Beiträge**, Bonn, **48**:167-177.

PESSÔA, L.M. & STRAUSS, R.E., 1999. Cranial size and shape variation, pelage and bacular morphology, and subspecific differentiation in spiny rats, *Proechimys albispinus* (Is. Geoffroy, 1838), from northeastern Brazil. **Bonner Zoologische Beiträge**, Bonn, **48**:231-243.

PESSÔA, L.M. & REIS, S.F., 2002 *Proechimys albispinus*. **Mammalian Species**, Northampton, **693**:1-3.

PESSÔA, L.M.; CORRÊA, M.M.O.; BITENCOURT, E. & REIS, S.F., 2005. Chromosomal characterization of taxa of the genus *Trinomys* Thomas, 1921, (Rodentia: Echimyidae) in the states of Rio de Janeiro and São Paulo. **Arquivos do Museu Nacional**, Rio de Janeiro, **63**(1):162-168.

REIS, S.F. & PESSÔA, L.M., 1995. *Proechimys albispinus minor*, a new subspecies from the state of Bahia, northeastern Brazil (Rodentia: Echimyidae). **Zeitschrift für Säugetierkunde**, Jena, **60**:181-190.

SMITH, M.F. & PATTON, J.L., 1988. Subspecies of pocket gophers: causal bases for geographic variation in *Thomomys bottae*. **Systematic Zoology**, Washington, **37**:163-178.

SOKAL, R.R. & ROHLF, F.J., 1981. **Biometry**. Second Edition. San Francisco: Freeman. 887p.

YONENAGA-YASSUDA, Y.; SOUZA, M.J.; KASAHARA, S.; L'ABBATE, M. & CHU, H.T., 1985. Supernumerary system in *Proechimys iheringi iheringi* (Rodentia, Echimyidae), from the state of São Paulo, Brazil. **Caryologia**, Florence, **38**:179-194.

ZANCHIN, N.I.T., 1988. **Estudos cromossômicos em orizomíneos e equimídeos da Mata Atlântica.** Msc. Dissertation, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre.